Group Analysis with AFNI Programs

- **Introduction**
  - ★ Most of the material and notations are from Doug Ward's manuals for the programs **`3dttest`**, **`3dANOVA`**, **`3dANOVA2`**, **`3dANOVA3`**, and **`3dRegAna,`** and from Gang Chen's recent modifications and documentation.
  - ★ Documentation available with the AFNI distribution
    - ➥ Lots of stuff (theory, examples) therein
  - ★ Software and documentation files are based on these books:
    - ➥ *Applied Linear Statistical Models* by Neter, Wasserman, and Kutner (4th edition)
    - ➥ *Applied Regression Analysis* by Draper and Smith (3rd edition)
  - ★ General steps
    - ➥ Smoothing (**`3dmerge -1blur_fwhm`**)
    - ➥ Normalization (**`3dcalc`**)
    - ➥ Deconvolution/Regression (**`3dDeconvolve`**)

    Individual subjects' analyses

    - ➥ Co-registration of individual analyses to common "space" (**`adwarp -dxyz`**)
    - ➥ Group analysis (**`3dttest`**, **`3dANOVA`**, …)

    Today's topic

    - ➥ Post-analysis (**`AlphaSim`**, conjunction analyses, …)
    - ➥ Interpretation and Thinking

- **Data Preparation: Spatial Smoothing**
  - ★ Spatial variability of both FMRI activation and the Talairach transform (the common space) can result in little or no overlap of function between subjects.
    - ➥ Data smoothing is used to reduce this problem.
      - ➪ Leads to loss of spatial resolution, but that is a price to be paid with the Talairach transform (or any current technique that does inter-subject anatomical alignments)
    - ➥ In principle, smoothing should be done on time series data, before data fitting (*i.e.*, before **`3dDeconvolve`** or **`3dNLfim`**, etc.)
      - ➪ Otherwise one has to decide on how to smooth statistical parameters.
        - In statistical data sets, each voxel has a multitude of different parameters associated with it like a regression coefficient, *t*-statistic, *F*-statistic, etc.
        - Combining some statistical parameters across voxels would result in parameters with unknown distributions
      - ➪ It is OK to blur percent signal change values that come out of the regression analysis, since these numbers depend linearly on the input data (unlike the *F*- and *t*-statistics)
      - ➪ Blurring in 3D is done using **`3dmerge`** with the **`-1blur_fwhm`** option
      - ➪ Blurring on the surface is done with program **`SurfSmooth`**

- **Data Preparation: Parameter Normalization**
  - ★ Parameters quantifying activation must be normalized before group comparisons.
    - ➥ FMRI signal amplitude varies for different subjects, runs, scanning sessions, regressors, image reconstruction software, modeling strategies, etc.
  - ★ Amplitude measures (regression coefficients) can be turned to percent signal change from baseline (do it before the individual analysis in **`3dDeconvolve`**).
    - ➥ Equations to use with **`3dcalc`** to calculate percent signal change
      - ⇨ 100 b_i / b_0 (basic formula)
      - ⇨ 100 $b_i / b_0 * c$ (mask out the outside of the brain)

        b_i = coefficient for regressor i (output from **`3dDeconvolve`**)

        b_0 = baseline estimate (output from **`3dTstat -mean`**)

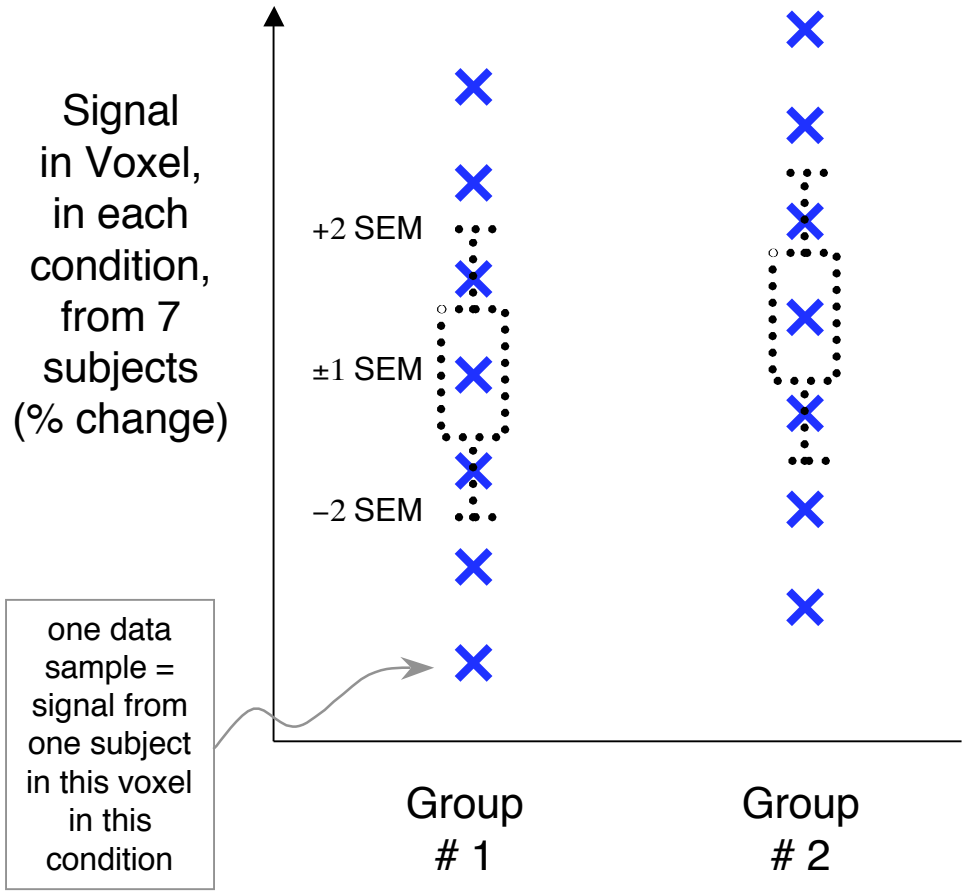
        c = threshold value generated from running **`3dAutomask -dilate`**
    - ➥ This will be included into **`3dDeconvolve`** in a future release
  - ★ Other normalization methods, such as z-score transformations of statistics, can also be used.

- **Data Preparation: Co-Registration (AKA "Spatial Normalization")**
  - ★ Group analyses are performed on a voxel-by-voxel basis
  - ★ All data sets used in the analysis must be aligned and defined over the same spatial domain.
    - ➥ Talairach domain for volumetric data
      - ➪ Landmarks for the transform are set on high-res. anatomical data using AFNI
      - ➪ Functional data volumes are then transformed using AFNI interactively or **`adwarp`** from command line (use option **`-dxyz`** with about the same resolution as EPI data — do ***not*** use the default 1 mm resolution!)
    - ➥ Standard meshes and spherical coordinate system for surface data
      - ➪ Surface models of the cortical surface are warped to match a template surface using Caret/SureFit (http://brainmap.wustl.edu) or FreeSurfer (http://surfer.nmr.mgh.harvard.edu)
      - ➪ Standard-mesh surface models are then created with **`SUMA`** (http://afni.nimh.nih.gov/ssc/ziad/SUMA) to allow for node-based group analysis using AFNI's programs
    - ➥ Once data is aligned, analysis is carried out voxel-by-voxel or node-by-node
      - ➪ The percent signal change from each subject in each task/stimulus state are usually the numbers that will be compared and contrasted
      - ➪ Resulting statistics (voxel-wise or node-wise) can then be displayed in AFNI and/or SUMA

- **Overview of Statistical Testing of Group Datasets with AFNI programs**
  - ★ Parametric Tests:
    - ➥ Assume data are normally distributed (Gaussian)
      - ⇨ **`3dttest`** (paired, unpaired)
      - ⇨ **`3dANOVA`** (or **`3dANOVA2`** or **`3dANOVA3`**)
      - ⇨ **`3dRegAna`** (regression, unbalanced ANOVA, ANCOVA)
      - ⇨ **`GroupAna`** = Matlab script for one-, two-, three-, four- and five- way ANOVA
  - ★ Non-parametric analyses:
    - ➥ No assumption of normality
    - ➥ Tends to be less sensitive to outliers (more robust)
      - ⇨ **`3dWilcoxon`** (~*t*-test paired)
      - ⇨ **`3dMannWhitney`** (~*t*-test unpaired)
      - ⇨ **`3dKruskalWallis`** (~**`3dANOVA`**)
      - ⇨ **`3dFriedman`** (~**`3dANOVA2`**)
      - ⇨ Permutation test
    - ➥ Less sensitive and less flexible than parametric tests
    - ➥ In practice, seems to make little difference
      - ⇨ Probably because number of datasets and subjects is usually small (hard to tell if data is non-Gaussian when only have a few sample points)

- ***t*-Tests** [starting easy, but contains most of the ideas]
  - ★ Program **`3dttest`**
    - ➥ Used to test if the mean of a set of values is significantly different from a constant (usually 0) or the mean of another set of values.
  - ★ Assumptions
    - ➥ Values in each set are normally distributed
    - ➥ Equal variance in both sets
    - ➥ Values in each set are independent ⇒ *unpaired* *t*-test
    - ➥ Values in each set are dependent ⇒ *paired* *t*-test
  - ★ Example: 20 subjects are tested for the effects of 2 drugs *A* and *B*
    - ➥ Case 1: 10 subjects were given drug *A* and the other 10 subjects given drug *B*.
      - ➪ Unpaired *t*-test is used to test: $m_A = m_B$? (mean response is different?)
      - ➪ Equivalent to one-way ANOVA with between-subjects design of equal sample size ⇒ can also run **`3dANOVA`** (treating subjects as multiple measurements)
    - ➥ Case 2: 20 subjects were given both drugs at different times.
      - ➪ Paired *t*-test is used to test: $m_A = m_B$?
    - ➥ Case 3: 20 subjects were given drug *A*.
      - ➪ *t*-test is used to test if drug effect is significant at group level: $m_A = 0$?

Unpaired 2 Sample *t*-Test: Cartoon Data



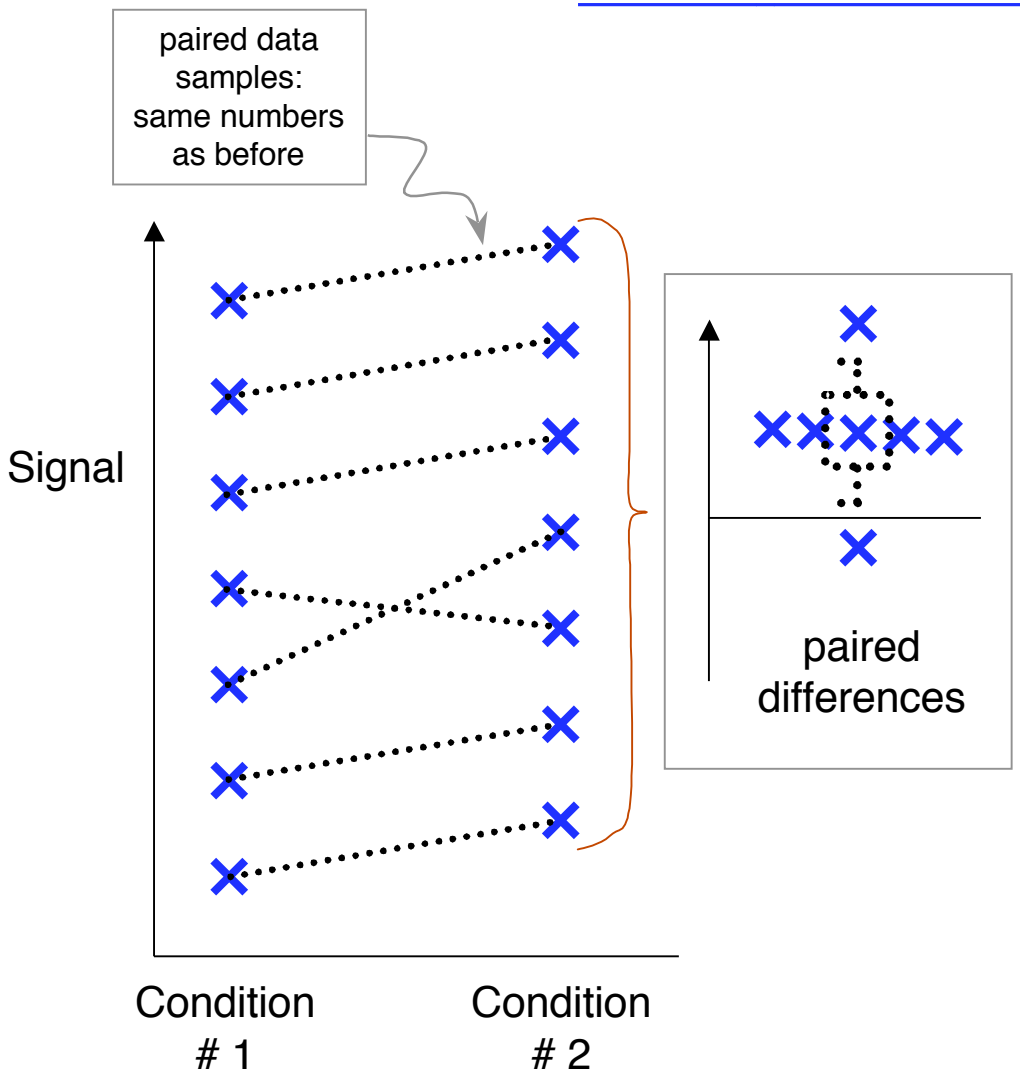
- Condition = some way to categorize data (*e.g.*, stimulus type, drug treatment, day of scanning, subject type, …)

- SEM = Standard Error of the Mean = standard deviation of sample divided by square root of number of samples
  = estimate of uncertainty in sample mean

- Unpaired *t*-test determines if sample means are "far apart" compared to size of SEM
  - *t* statistic is difference of means divided by SEM

- Not significantly different!

Paired *t*-Test: Cartoon Data



- Paired means that samples in different conditions should be linked together (*e.g.*, from same subjects)

- Test determines if differences between conditions in each pair are "large" compared to SEM of the differences

- Paired test can detect systematic *intra*-subject differences that can be hidden in *inter*-subject variations

- Lesson: properly separating ***inter***-subject and ***intra***-subject signal variations can be very important!

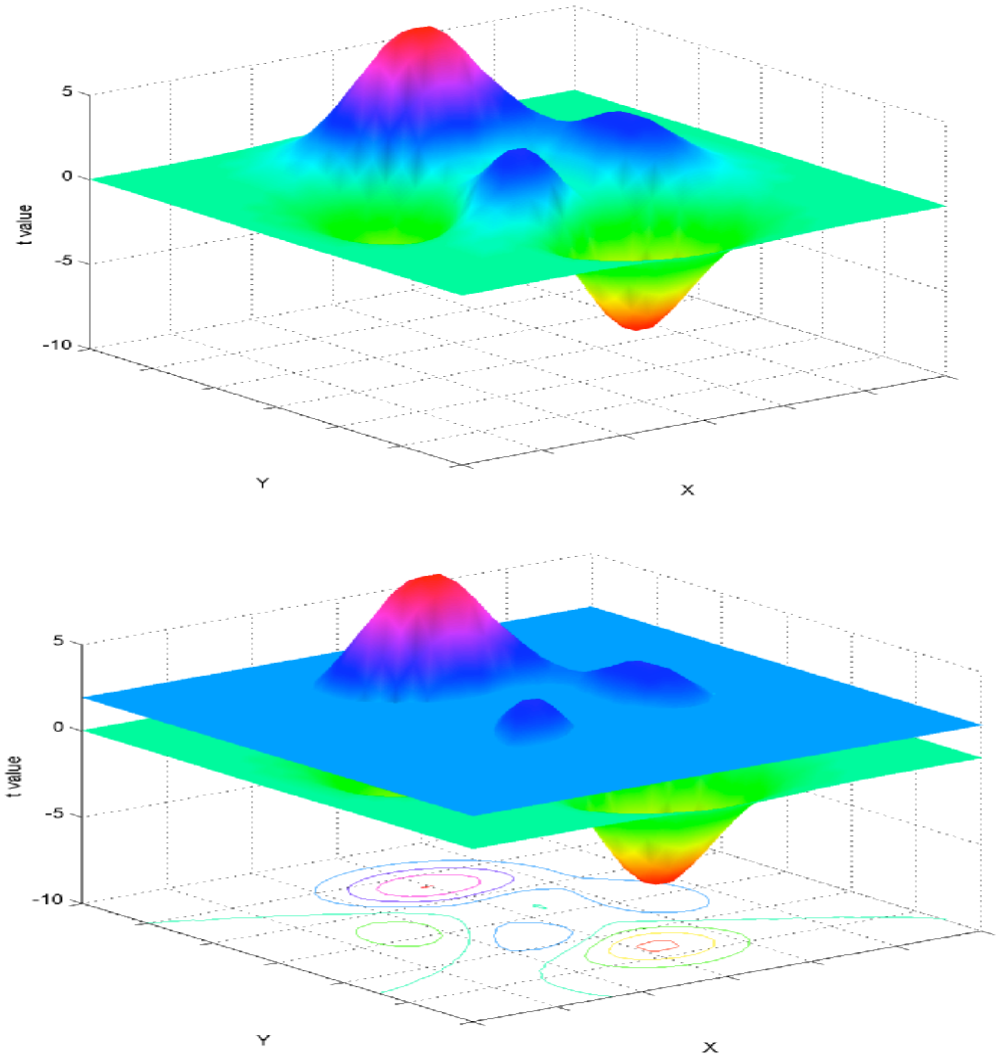
- Significantly different!
- Condition #2 > #1, per subject

• Basics: Null hypothesis significance testing (NHST)

- ★ *Main function of statistics is to get more information into the data*
- ★ Null and alternative hypotheses
  - ➥ **H_0**: nothing happened vs. **H_1**: something happened
- ★ Dichotomous decision
  - ➥ Rejecting H_0 at a significant level α (e.g., 0.05)
  - ➥ Subtle difference

    Traditional: Hypothesis holds until counterexample occurs;

    Statistical: discovery holds when a null hypothesis is rejected with some statistical confidence
  - ➥ Topological landscape vs. binary world



• Basics: Null hypothesis significance testing (NHST)

★ Dichotomous decision

➥ **Conditional probability** $P(\text{reject } H_0 \mid H_0) = \alpha \neq P(H_0)$ (unknown)!

➥ 2 types of errors and power

➢ Type I error = $\alpha = P(\text{reject } H_0 \mid H_0)$ aka false +

➢ Type II error = $\beta = P(\text{accept } H_0 \mid H_1)$ aka false -

➢ Power = $P(\text{accept } H_1 \mid H_1) = 1 - \beta$

Justice System: Trial

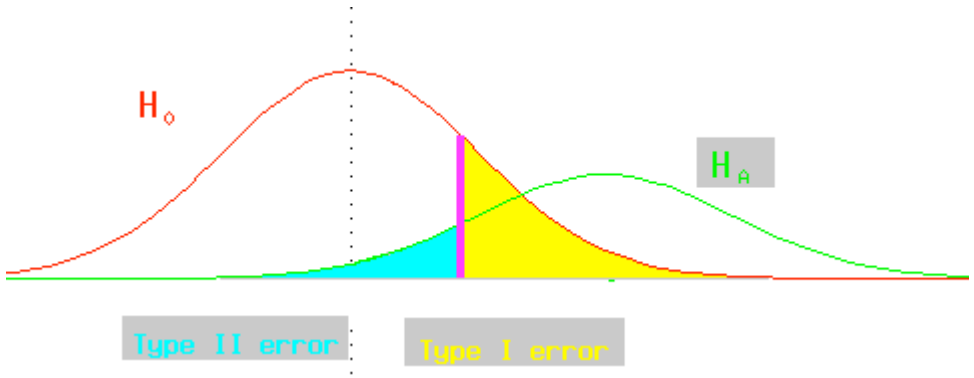
	Defendant Innocent	Defendant Guilty
Reject Presumption of Innocence (Guilty Verdict)	**Type I Error**	**Correct**
Fail to Reject Presumption of Innocence (Not Guilty Verdict)	**Correct**	**Type II Error**

Statistics: Hypothesis Test

	H_0 True	H_0 False
Reject H_o	**Type I Error**	**Correct**
Fail to Reject H_0	**Correct**	**Type II Error**

• Basics: Null hypothesis significance testing (NHST)

↙Compromise and strategy



➢Lower type II error under fixed type I error

❖Control false + while gaining as much power as possible

➢Check **efficiency** (power) of design with **`RSFgen`** before scanning

↙Typical misinterpretations*)

➢Reject H_0 --> Prove or confirm a theory (alternative hypothesis)! (wrong!)

➢$P(\text{ reject } H_0 \mid H_0) = P(H_0)$ (wrong!)

➢$P(\text{ reject } H_0 \mid H_0)$ = Probability if the experiment can be reproduced (wrong!)

*) *Cohen, J., "The Earth Is Round (p < .05)" (1994), American Psychologist, 49, 12 997-1003*

• Basics: Null hypothesis significance testing (NHST)

★ Controversy: Are humans cognitively good intuitive statisticians?

★ Quiz: HIV prevalence = 10^{-3}, false + of HIV test = 5%, power of HIV test ~ 100%.

➥ P(HIV+ I test+) = ?

$$P(HIV+ \mid test+) = \frac{P(test+ \mid HIV+)P(HIV+)}{P(test+ \mid HIV+)P(HIV+) + P(test+ \mid HIV-)P(HIV-)} = \frac{1.0 \times 10^{-3}}{1.0 \times 10^{-3} + 0.05 \times (1 - 10^{-3})} \approx 0.02$$

★ Keep in mind

➥ Better plan than sorry: Spend more time on experiment design (power analysis)

➥ More appropriate for detection than sanctification of a theory

➢ Modern phrenology?

➥ Try to avoid unnecessary overstatement when making conclusions

➥ Present graphics and report % signal change, standard deviation, confidence interval, …

➥ Replications are the best strategy on induction/generalization

➢ Group analysis

★ Quiz

A researcher tested the null hypothesis that two population means are equal (H_0: $\mu_1 = \mu_2$). A *t*-test produced p=0.01. Assuming that all assumptions of the test have been satisfied, which of the following statements are true and which are false? Why?

1. There is a 1% chance of getting a result even more extreme than the observed one when H_0 is true.
2. There is a 1% likelihood that the result happened by chance.
3. There is a 1% chance that the null hypothesis is true.
4. There is a 1% chance that the decision to reject H_0 is wrong.
5. There is a 99% chance that the alternative hypothesis is true, given the observed data.
6. A small *p* value indicates a large effect.
7. Rejection of H_0 confirms the alternative hypothesis.
8. Failure to reject H_0 means that the two population means are probably equal.
9. Rejecting H_0 confirms the quality of the research design.
10. If H_0 is not rejected, the study is a failure.
11. If H_0 is rejected in Study 1 but not rejected in Study 2, there must be a moderator variable that accounts for the difference between the two studies.
12. There is a 99% chance that a replication study will produce significant results.
13. Assuming H_0 is true and the study is repeated many times, 1% of these results will be even more inconsistent with H_0 than the observed result.

Adapted from Kline, R. B. (2004). Beyond significance testing. Washington, DC: American Psychological Association (pp. 63-69). Dale Berger, CGU 9/04

★ *Hint: Only 2 statements are true*

- **1-Way ANOVA**
  - ★ Program **3dANOVA**
    - ➥ Determine whether treatments (levels) of a single factor (independent parameter) has an effect on the measured response (dependent parameter, like FMRI percent signal change due to some stimulus).
    - ➥ Examples of *factor*: subject type, task type, task difficulty, drug type, drug dosage, *etc. Only when groups must be different across factor levels*
    - ➥ Within a factor are *levels*: different sub-categorizations
      - ➪ Example: factor=subject type; level 1=normals, level 2=patients with mild symptoms, level 3=patients with severe symptoms
    - ➥ The various AFNI ANOVA programs differ in the number of factors they allow: **3dANOVA** allows 1 factor, comprising up to 100 levels
  - ★ Assumptions
    - ➥ Values are normally distributed
    - ➥ No assumptions about relationship between dependent and independent variables (e.g., not necessarily linear)
    - ➥ Independent variables are qualitative
  - ★ Can also use **3dttest** if there are only two levels
    - ➥ The 1-way **3dANOVA** analysis is a generalization to multiple levels of an *unpaired* **3dttest** (for generalization of *paired*, wait for **3dANOVA2**)
  - ★ Example: *r* different types of subjects performed the same task in the scanner

Data from Voxel *V*	**Factor levels** (*e.g.*, subject types)			
	1	2	…	r
Measurements (*e.g.*, percent signal change)	$Y_{1,1}$	$Y_{2,1}$	…	$Y_{r,1}$
	$Y_{1,2}$	$Y_{2,2}$	…	$Y_{r,2}$
	…	…	…	…
	$Y_{1,n1}$	…	…	…
		…	…	$Y_{r,nr}$
		$Y_{2,n2}$	…	

e.g., Subjects are multiple measurements within each level

Null Hypothesis: H_0 : $m_1 = m_2 = \ldots = m_r$

i.e., subject type has no effect on mean signal in this voxel

Alternative Hypothesis: H_a : *not all* m_i *are equal*

i.e., at least one subject type had a different mean FMRI signal

- **`3dANOVA`** is effectively a generalization of the unpaired *t*-test to multiple columns of data (a further refinement will be introduced with **`3dANOVA3`**)
  - As such, **`3dANOVA`** is probably not appropriate when comparing results of different tasks on the same subjects (need a generalization of the paired *t*-test: **`3dANOVA2`**)

- **ANOVA: Which levels had an effect or were different from one another?**
  - Usually, just knowing that there is a *main effect* (some of the means are different, but no information about *which* ones) isn't enough, so there is a number of options to let you look for more detail
  - Which treatment means (m_i) are ≠ 0 ?
    - ➥ *e.g.*, is the response of subjects in level #3 different from 0 ?
    - ➥ *t*-statistic with option **`-mean`** in **`3dANOVA`**
    - ➥ Similar to using **`3dttest -base1 0`** (single sample test) to test only the data from those subjects
  - Which treatment means are different from each other ?
    - ➥ *e.g.*, is the response of subjects in level #3 different from those in level #2 ?
    - ➥ *t*-statistic with option **`-diff`** in **`3dANOVA`**
    - ➥ Similar to using **`3dttest`** (unpaired) between the data from these sets of subjects
  - Which linear combination of means (*contrasts*) are ≠ 0 ?
    - ➥ *e.g.*, is the average response of subjects in level #1 different from the combined average of subjects in levels #2 and #3 ?
    - ➥ *t*-statistic with option **`-contr`** in **`3dANOVA`**

- **Nomenclature**
  - ★ Random factor
    - ➥ Typically subject in fMRI
    - ➥ Factor levels are of no particular interest
  - ★ Fixed factor
    - ➥ Typically non-subject factors
    - ➥ Factor levels are of particular interest
  - ★ Within-subject (repeated-measures) factor
    - ➥ Every subject of factor B performs all levels of a particular factor A
      - ➪ Crossed design AxB: A - task; B - subject
  - ★ Between-subjects factor
    - ➥ Each subject of factor B belongs to one level of factor A
      - ➪ Nested design B(A): A - gender; B - subject
  - ★ Mixed design (not mixed-effects model)
    - ➥ Have both within-subject and between-subjects factors
      - ➪ BxC(A): A - gender; B - task; C - subject
  - ★ Mixed-effects model
    - ➥ In multi-way ANOVA with both random and fixed factors (almost all cases)

- **2-Way ANOVA**: **test for effects of two independent factors on measurements**
  - ★ This is a *fully crossed* analysis: all combinations of factor levels are measured
    - ➥ In particular, if one factor is "subject", then all subjects are tested in all levels of the other factor
    - ➥ Program is limited to *balanced designs*: Must have same number of measurements in each "cell" (combinations of factor levels)
  - ★ Example: Stimulus type for factor A and subject for factor B
    - ➥ Each subject is a level within factor B (1 measurement per cell)
    - ➥ This is a *fixed effect* × *random effect* model = "mixed effect" model
  - ★ Example: Stimulus type for factor A, stimulus day for factor B
    - ➥ With one fixed subject, for a longitudinal study (*e.g.*, training between scan days)
    - ➥ This also is a *fixed effect* × *fixed effect* model
    - ➥ With multiple subjects go with **`3dANOVA3`** with subject as the third (random) factor

see next pages for description of ***fixed*** and ***random*** *effects*

- *Random effects* factor = differences between levels in this factor are modeled as random fluctuations
  - ★ Useful for categories not under experimenter's control or observation
  - ★ In FMRI, is especially useful for subjects; a good rule is

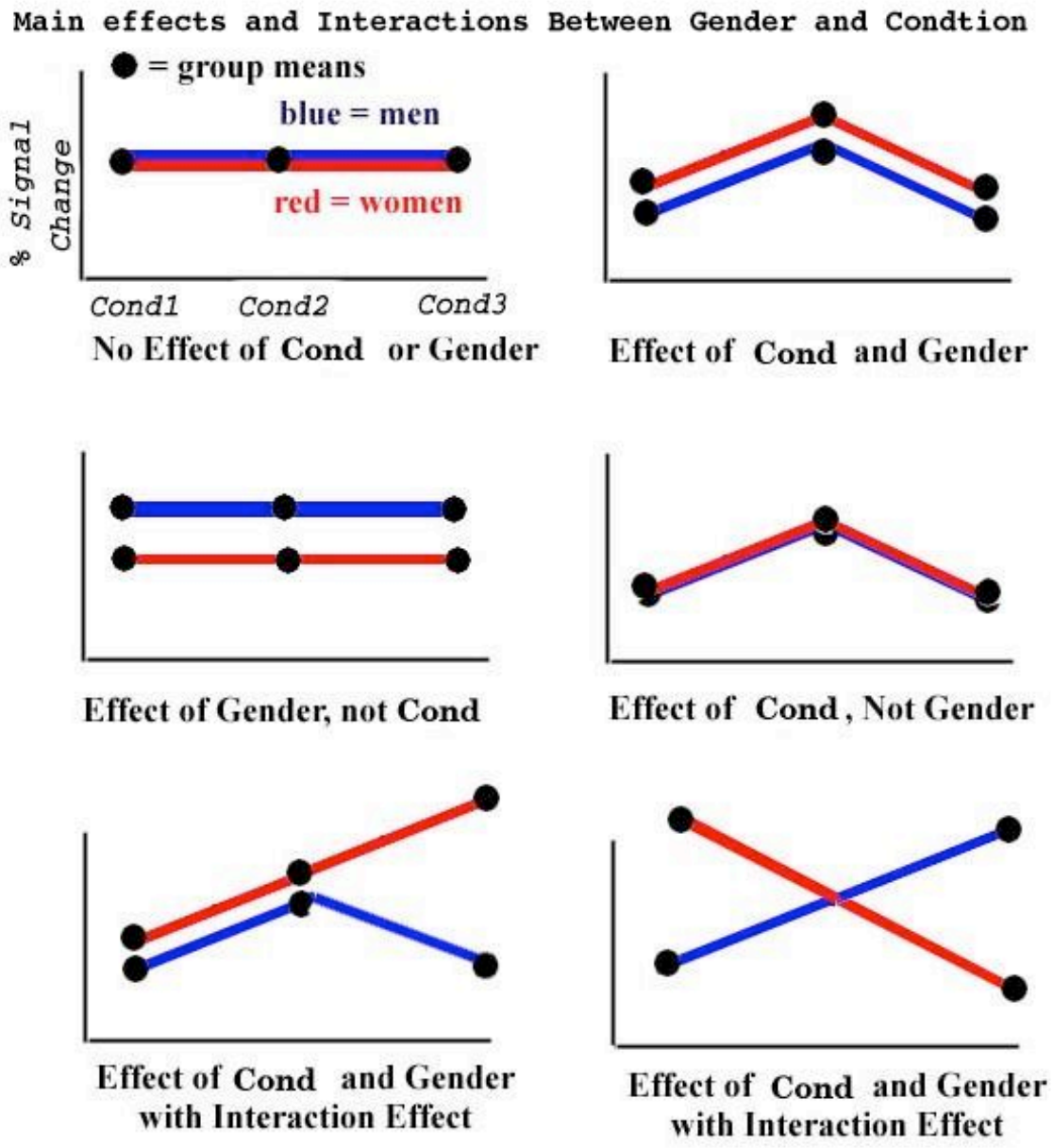
    treat subjects as a separate random effects factor rather than as multiple independent measurements inside fixed-effect factors
  - ★ In such a case, usually have 1 measurement per cell (each cell is the combination of a level from the other factor with 1 subject)
    - ➥ This is sometimes called a "repeated measures ANOVA", when we have multiple measurements on each subject (in this case, across different stimulus classes)
  - ★ Treating subjects as a random factor in a fully crossed analysis is a generalization of the paired *t*-test
    - ➥ intra-subject and inter-subject data variations are modeled separately
    - ➥ which can let you detect small intra-subject changes due to the fixed-effect factors that might otherwise be overwhelmed by larger inter-subject fluctuations
  - ★ Main effect for a random effects factor tests if fluctuations among levels in this factor have additional variance above that from the other random fluctuations in the data
    - ➥ *e.g.*, Are inter-subject fluctuations bigger than intra-subject fluctuations?
    - ➥ Not usually very interesting when random factor = subject
  - ★ It is hard to think of a good FMRI example where both factors would be random
  - ★ **`3dANOVA2`**: Usually have 1 fixed factor and 1 random factor = *mixed effects* analysis

- *Fixed effects* factor = differences between levels in this factor are modeled as deterministic differences in the mean measurements (as in **`3dANOVA`** and **`3dttest`**)
  - ★ Useful for most categories under the experimenter's control or observation
  - ★ Allows same type of statistics as **`3dANOVA`**:
    - factor main effect (are all the mean activations of each level in this factor the same?)
    - differences between level pairs (*e.g.*, level #2 same as #3?)
    - more complex contrasts (*e.g.*, average of levels #1 and #2 same as level #3?)
  - ★ If two or more factors are modeled as fixed effects:
    - ➥ Can also test for *interaction* between fixed factors
      - ➪ Are there any combinations of factor levels whose means "stick out" [*e.g.*, mean of cell #(A_1,B_2) differs from (#A_1 mean)+(#B_2 mean)]?
      - ➪ Example: A=stimulus type, B=drug type; then cell #(A_1,B_2) is FMRI response (in each voxel) to stimulus #1 and drug #2
      - ➪ Interaction test would determine if any individual combination of drug type and stimulus type was abnormal
        - *e.g.*, if stimulus #1 averages a high response, and drug #2 averages no effect on response, but when together, value in cell #(A_1,B_2) averages small
        - *i.e.*, Effect of one factor (stimulus) depends on level of other factor (drug)
        - no interaction means the effects of the factors are always just additive
    - ➥ Inter-factor contrasts can then be used to test individual combinations of cells to determine which cell(s) the interaction comes from

• Basics: ANOVA

★ More terminology

- **Main effect**: general info regarding all levels of a factor
- **Simple effect**: specific info regarding a factor level
- **Interaction**: mutual/reciprocal influence among 2 or more factors; parallel or not?
- Disordinal interaction: differences reverse sign
- Ordinal interaction: one above another
- **Contrast**: comparison of 2 or more simple effects; coefficients add up to 0
- **General linear test**



Main effects and interactions in 2-way mixed ANOVA

	Data from Voxel *V*	**factor *B* levels** (*e.g.*, subject)			
		1	**2**	**…**	**b**
Factor *A* levels (*e.g.*, stimulus type, drug dose, ...)	**1**	Y_{111} Y_{112} … Y_{11n}	Y_{121} Y_{122} … Y_{12n}	… … … …	Y_{1b1} Y_{1b1} … Y_{1bn}
	2	Y_{211} Y_{212} … Y_{21n}	Y_{221} Y_{222} … Y_{22n}	… … … …	Y_{2b1} Y_{2b1} … Y_{2bn}
	…	.	.	.	.
	a	Y_{a11} Y_{a12} … Y_{a1n}	Y_{a21} Y_{a22} … Y_{a2n}	… … … …	Y_{ab1} Y_{ab1} … Y_{abn}

NOTE WELL: Must have same number of observations ("*n*") in each cell

Can use **`3dRegAna`** if you don't have the same number of values in each cell (program usage is much more complicated)

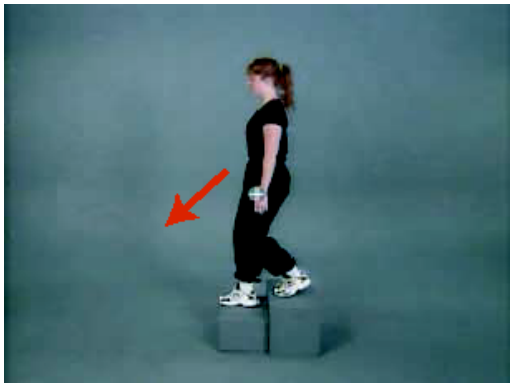
- **3-Way ANOVA: `3dANOVA3`** (again, balanced designs only)
  - ★ Read the manual first and understand what options are available
    - ➥ It is important to understand 2-way ANOVA before moving up to the big time show!
  - ★ Has several fixed effects and random effects combinations
  - ★ Has *nested design* (vs. fully crossed design)
    - ➥ Nested design is for use when you have 2 fixed effects factors and 1 random effects factor where the subjects for the random effects factor depend on one of the fixed effect factors; example:
      - ➪ factor A = subject type; level #1=normal, #2=genotype Q, #3=genotype R
      - ➪ factor B = stimulus type; levels #1–4=different types of videos
      - ➪ factor C = subject; levels #1–10 = 30 different subjects, 10 in each of the factor A levels; C is "nested" inside A
    - ➥ Nested design is a mixture of unpaired and paired tests
      - ➪ Will be like "paired" for tests across stimulus type (factor B levels)
      - ➪ Will be like "unpaired" across subject types (factor A levels)
    - ➥ Fully crossed design is when the subjects are common across the other factors
      - ➪ As was said before, un-nested design is a generalization of paired *t*-test
    - ➥ Treating the subjects correctly is a crucially important decision

- **Group Analysis**: **`3dANOVA3`**
  - ★ Designs
    - ➥ Three-way between-subjects (**`type 1`**)
    - ➥ Two-way within-subject (**`type 4`**): Crossed design AXBXC
      - ➢ Generalization of paired *t*-test
      - ➢ One group of subjects
      - ➢ Two categorizations of conditions: A and B
    - ➥ Two-way mixed (**`type 5`**): Nested design BXC(A)
      - ➢ Two or more groups of subjects (Factor A): subject classification, e.g., gender
      - ➢ One category of condition (Factor B)
      - ➢ Nesting: balanced (i.e. 12 male, 12 female subjects)
  - ★ Output
    - ➥ Main effect (**`-fa and -fb`**) and interaction (**`-fab`**): *F*
    - ➥ Contrast testing
      - ➢ 1st order: **`-amean, -adiff, -acontr, -bmean, -bdiff, -bcontr`**
      - ➢ 2nd order: **`-abmean, -aBdiff, -aBcontr, -Abdiff, -Abcontr`**
      - ➢ 2 values per contrast : % and *t*

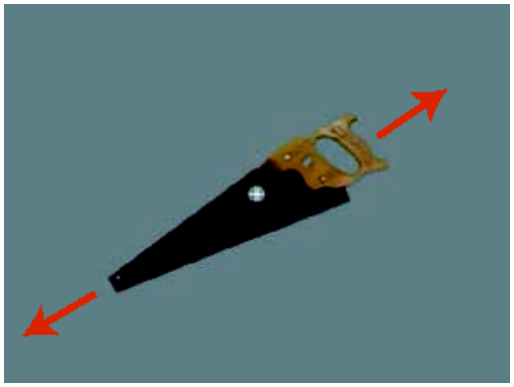
- **3dANOVA3**: A test case
  - ★ Michael S. Beauchamp, Kathryn E. Lee, James V. Haxby, and Alex Martin, *fMRI Responses to Video and Point-Light Displays of Moving Humans and Manipulable Objects*, Journal of Cognitive Neuroscience, **15**: 991-1001 (2003).
  - ★ Purpose is to study the organization of brain responses to different types of complex visual motion (the 4 levels within factor A) from 9 subjects (the levels within factor B)
  - ★ Data from 3 of the subjects, and scripts to process it with AFNI programs, are available in AFNI HowTo #5 (hands-on)
    - ➥ Available for download at the AFNI web site: **http://afni.nimh.nih.gov/afni/doc/howto/**
    - ➥ If you want *all* the data, it is at the FMRI Data Center at Dartmouth: **http://www.fmridc.org**
    - ➥ Or at least, it **should** be (but they haven't posted it yet for some reason)

- **Stimuli: Video clips of the following**

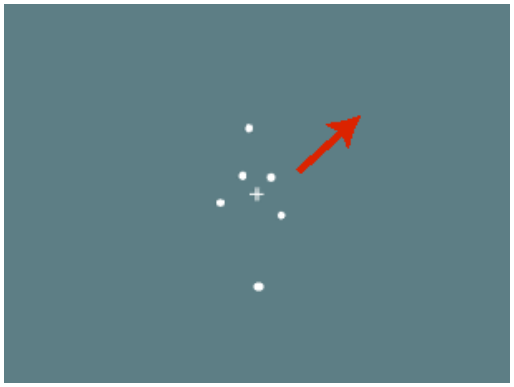
Human whole-body motion (HM)



Tool motion (TM)



Human point motion (HP)



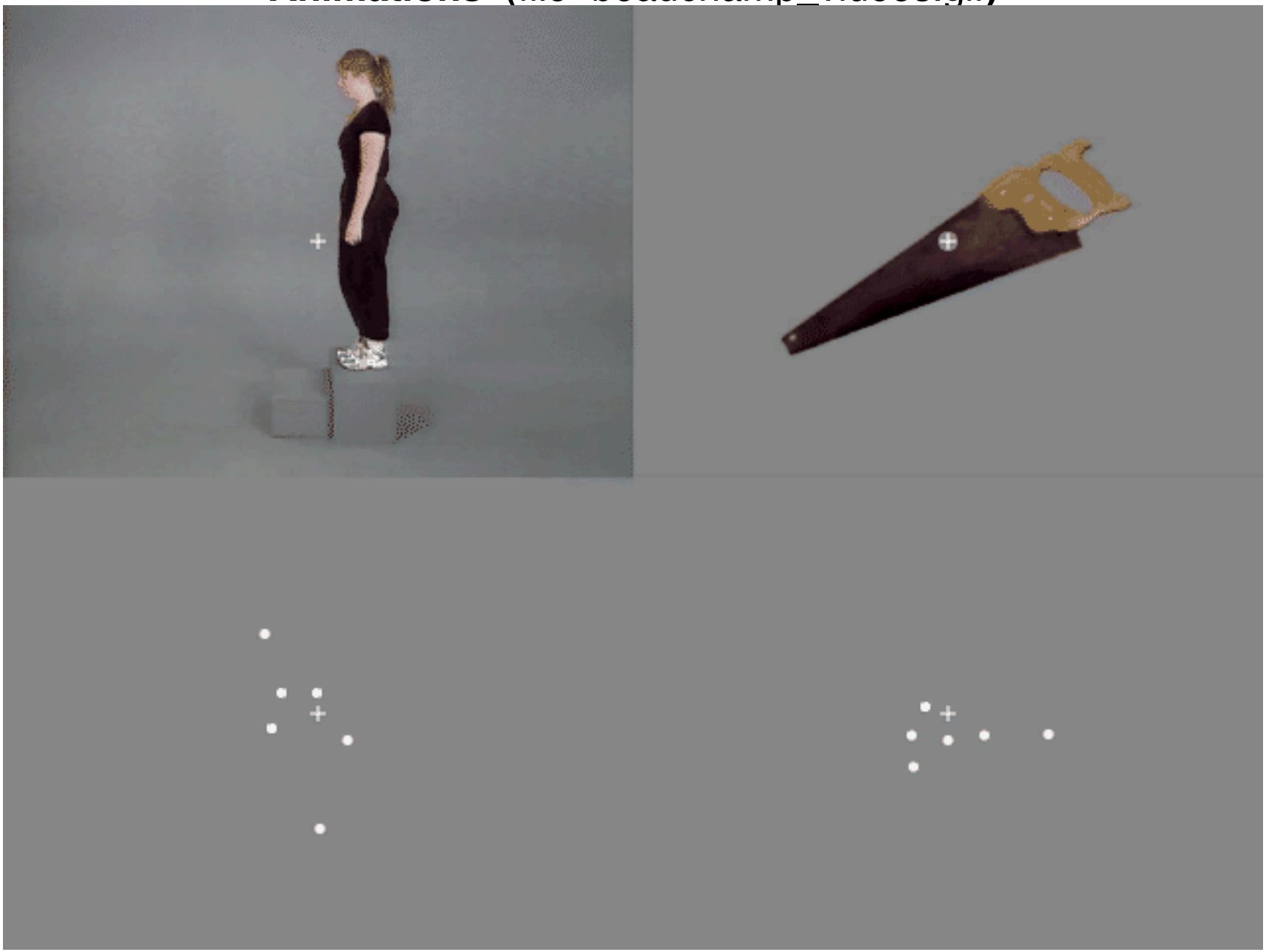
From Figure 1
Beauchamp et al. 03

Tool point motion (TP)

Hypotheses to test:

- Which areas are differentially activated by any of these stimuli (main effect)?
- Which areas are differentially activated for point motion versus natural motion? (type of image)
- Which areas are differentially activated for human-like versus tool-like motion? (type of motion)

Animations (file=beauchamp_videos.gif)



- **Data Processing Outline**
  - ★ Image registration with **`3dvolreg`**
  - ★ Images smoothed (4 mm FWHM) with **`3dmerge`**
  - ★ IRF for each of the 4 stimuli were obtained using **`3dDeconvolve`**
  - ★ Regressor coefficients (IRFs) were normalized to percent signal change (using **`3dcalc`**)
  - ★ An average activation measure was obtained by averaging IRF amplitude (using **`3dTstat`**)
    - ➥ These activation measures will be the measurements in the ANOVA table
      - ➪ After each subject's results are warped to Talairach coordinates, using **`adwarp`** program

• Group Analysis: Example

★ Script

```
3dANOVA3 -type 4  -alevels 2  -blevels 2  -clevels 8 \

-dset 1 1 1 ED_TM_irf_mean+tlrc \
-dset 1 2 1 ED_TP_irf_mean+tlrc \
-dset 2 1 1 ED_HM_irf_mean+tlrc \
-dset 2 2 1 ED_HP_irf_mean+tlrc \
…
-adiff    1  2 TvsH1 \  (indices for difference)
-acontr   1 -1 TvsH2 \  (coefficients for contrast)
-bdiff    1  2 MvsP1 \
-aBdiff   1  2 : 1 TMvsHM \  (indices for difference)
-aBcontr  1 -1 : 1 TMvsHM \  (coefficients for contrast)
-aBcontr -1  1 : 2 HPvsTP \
-Abdiff   1 : 1  2 TMvsTP \
-Abcontr  2 : 1 -1 HMvsHP \

-fa  ObjEffect \
-fb  AnimEffect \
-fab ObjXAnim \

-bucket Group
```

Model type, number of levels for each factor

Input for each cell in ANOVA table: totally 2X2X8 = 32

1st order Contrasts, paired *t* test

2nd order Contrasts, paired *t* test

Main effects & interaction *F* test; Equivalent to contrasts

Output: bundled

- **4 & 5-Way ANOVA: ready to rock-n-roll** (for the daring and intrepid)
  - ★ Interactive Matlab script (user-friendly)
  - ★ Can run both crossed and nested (*i.e.,* subject nested into gender) design
  - ★ Heavy duty computation + Matlab: expect to take 10s of minutes to hours
  - ★ Same script can also do **`ANOVA`**, **`ANOVA2`**, and **`ANOVA3`** analyses
  - ★ Includes contrast tests across all factors
  - ★ Balanced design with no missing data in most cases
    - ➥ Unbalanced design allowed with unequal number of subject across groups (e.g., unequal number of males and females). Much simpler than using **`3dRegAna`**

5 Types of 4-Way ANOVA

$A_F \times B_F \times C_F \times D_F$ All factors fixed; fully crossed	A,B,C,D=stimulus category, drug treatment, etc. All combinations of subjects and factors exist; Multiple subjects: treated as multiple measurements; One subject: longitudinal analysis
$A_F \times B_F \times C_F \times D_R$ Last factor random; fully crossed	A,B,C=stimulus category, etc. D=subjects, typically treated as random (more powerful than treating them as multiple measurements) Good for an experiment where each fixed factor applies to **all** subjects;
$B_F \times C_F \times D_R(A_F)$ Last factor random, and nested within the first (fixed) factor	A=subject class: genotype, sex, or disease B,C=stimulus category, etc. D=subjects nested within A levels
$B_F \times C_R \times D_F(A_F)$ Third factor random; fourth factor fixed and nested within the first (fixed) factor	A=stimulus type (*e.g.*, repetition number) B=another stimulus category (*e.g.*, animal/tool) C=subjects (a common set among all conditions) D=stimulus subtype (*e.g.*, perceptual/conceptual)
$C_F \times D_R(A_F \times B_F)$ Doubly nested! (The PSFB special)	A, B=subject classes: genotype, sex, or disease C=stimulus category, etc. D=subjects, random with two distinct factors dividing the subjects into finer sub-groups (*e.g.*, A=sex × B=genotype)

3 Design Types of 5-Way ANOVA

$A_F \times B_F \times C_F \times D_F \times E_F$ All factors fixed; fully crossed	A,B,C,D,E=stimulus category, drug treatment, etc. All combinations of subjects and factors exist; Multiple subjects: treated as multiple measurements; One subject: longitudinal analysis
$A_F \times B_F \times C_F \times D_F \times D_R$ Last factor random; fully crossed	A,B,C,D=stimulus category, etc. E=subjects, random Fully crossed design
$B_F \times C_F \times D_F \times E_R(A_F)$ Last factor random, and nested within the first (fixed) factor	A=subject class: group, genotype, sex, or disease B,C,D=stimulus category, etc. E=subjects nested within A levels

- A real example with 5-way mixed design (neural mechanism for category-selective response):
  - Factors
    - Task (between-subject): semantic decision, naming
    - Modality: visual, auditory
    - Format: verbal, nonverbal
    - Category: animal, tool
    - Subject (random)
  - 4 stimuli (2X2) for animal and tool - visual verbal = word, visual nonverbal = picture, auditory verbal = spoken, auditory nonverbal = sound
  - 4-way mixed design: Only 2 levels for all 3 within-subject factors: no concern for sphericity violation

- **Conjunction Junction: What's Your Function?**
  - ★ The program **`3dcalc`** is a general purpose program for performing logic and arithmetic calculations
    - ➥ Command line is of the format

```
3dcalc -a Dset1 -b Dset2 ... -expr "(a * b ...)"
```

values from **`Dset1`** are to be called '**`a`**' in **`-expr`**

mathematical expression combining input dataset values

    - ➥ Some expressions can be used to select voxels with values *v* meeting certain criteria:
      - ➪ Find voxels where $v \geq th$ and mark them with value=1

        expression = ***step (v – th)*** (result is 1 or 0)
      - ➪ In a range of values: $th_{min} \leq v \leq th_{max}$

        expression = ***step (v – th_{min}) * step (th_{max} - v)***
      - ➪ Exact value: $v = n$

        expression = **equals*(v – n)***
    - ➥ Create masks to apply to functional datasets
      - ➪ Two values both above threshold (*e.g.,* active in both tasks; "conjunction")

        expression = ***step(v-A)*step(w-B)***

- **Regression Analysis: `3dRegAna`**
  - ★ Simple linear regression:
    - ➥ $Y = \beta_0 + \beta_1 X_1 + \varepsilon$
    - ➥ where Y represents the FMRI measurement (*i.e.*, percent signal change) and X is the independent variable (*i.e.*, drug dose)
  - ★ Multiple linear regression:
    - ➥ $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \ldots + \varepsilon$
  - ★ Regression with qualitative and quantitative variables (ANCOVA)
    - ➥ *i.e.*, drug dose (5mg, 12mg, 23mg, etc.) is quantitative while drug type (Nicotine, THC, Cocaine) or age group (young vs. old) or genotype is qualitative, and usually called dummy (or indicator) variable
  - ★ ANOVA with unequal sample sizes (with indicator variables)
  - ★ Polynomial regression:
    - ➥ $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_1^2 + \ldots + \varepsilon$
  - ★ Linear regression: model is a linear function of its unknowns β_i , *NOT* its independent variables X_i
  - ★ Not for fitting time series, use **`3dDeconvolve`** (or **`3dNLfim`**) instead

- *F*-test for Lack of Fit (*lof*)
  - ★ If multiple measurements are available (and they should be), a ***Lack Of Fit*** *(lof)* test is first carried out.
    - ➥ Hypothesis:

      H_0: $E(Y) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots, + \beta_{p-1} X_{p-1}$

      H_a: $E(Y) \neq \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots, + \beta_{p-1} X_{p-1}$

    - ➥ Hypothesis is tested by comparing the variance of the model's lack of fit to the measurement variance at each point (pure error).
    - ➥ If F_{lof} is significant then model is inadequate. STOP HERE.
      - ➪ Reconsider independent variables, try again.
    - ➥ If F_{lof} is insignificant then model appears adequate, so far.
    - ➥ It is important to test for the lack of fit:
      - ➪ The remainder of the analysis assumes an adequate model is used
      - ➪ You will not be visually inspecting the goodness of the fit for thousands of voxels!

- Test for Significance of Linear Regression
  - ★ This is done by testing whether additional parameters significantly improve the fit
    - ➥ For simple case

      $Y = \beta_0 + \beta_1 X_1 + \varepsilon$

      H_0: $\beta_1 = 0$

      H_1: $\beta_1 \neq 0$

    - ➥ For general case

      $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ \ldots + \beta_{q-1} X_{q-1} + \ \beta_q X_q + \ldots + \beta_{p-1} X_{p-1} + \varepsilon$

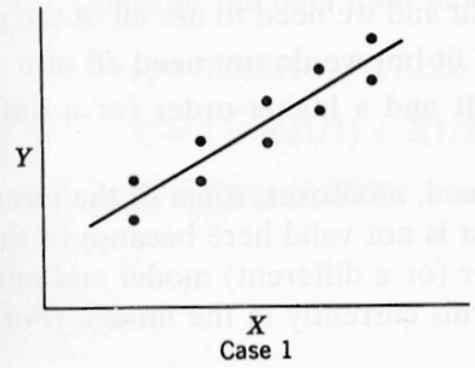
      H_0: $\beta_q = \beta_{q+1} = ... = \beta_{p-1} = 0$

      H_a: $\beta_k \neq 0$, for some $k,\ q \leq k \leq p-1$

    - ➥ *Freg i*s the *F*-statistic for determining if the Full model significantly improved on the reduced model
      - ➪ *NOTE:* This *F*-statistic is assumed to have a central *F*-distribution. This is **not** the case when there is a lack of fit

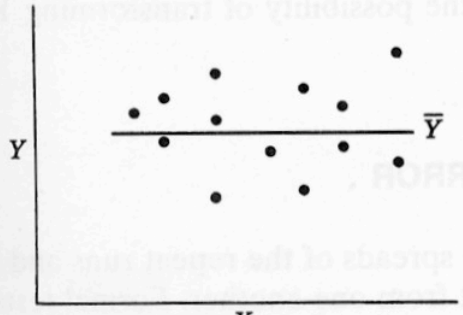
- **`3dRegAna`**: Other statistics
  - ★ How well does model fit data?
    - ➥ R^2 (coefficient of multiple determination) is the proportion of the variance in the data accounted for by the model $0 \leq R^2 \leq 1$.
    - ➥ *i.e.*, if $R^2 = 0.26$ then 26% of the data's variation about their mean is accounted for by the model. So this might indicate the model, even if significant, might not be that useful (depends on what use you have in mind)
      - ➪ Having said that, you should consider R^2 relative to the maximum it can achieve given the pure error which cannot be modeled. [*cf.* Draper & Smith, chapter 2].
  - ★ Are individual parameters β_k significant?
    - ➥ *t*-statistic is calculated for each parameter
    - ➥ helps identify parameters that can be discarded to simplify the model
  - ★ R^2 and *t*-statistic are computed for full (not reduced) model

Examples from Applied Regression Analysis by Draper and Smith (third edition)



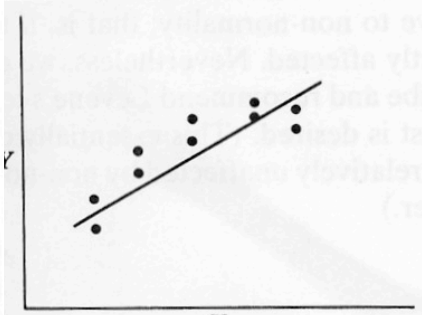
Case 1

Case 1:
(1) Try $Y = \beta_0 + \beta_1 X + \varepsilon$.
(2) No lack of fit.
(3) Significant linear regression.
(4) Use model $\hat{Y} = b_0 + b_1 X$.



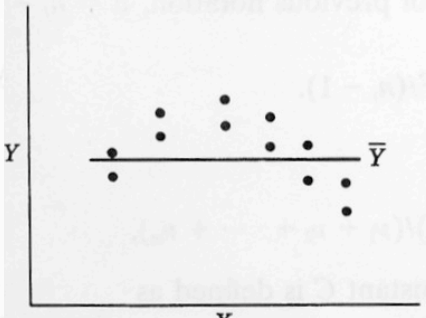
Case 2

Case 2:
(1) Try $Y = \beta_0 + \beta_1 X + \varepsilon$.
(2) No lack of fit.
(3) Linear regression not significant.
(4) Use model $\hat{Y} = \overline{Y}$.



Case 3

Case 3:
(1) Try $Y = \beta_0 + \beta_1 X + \varepsilon$.
(2) Significant lack of fit.
(3) Try model $Y = \beta_0 + \beta_1 X + \beta_{11} X^2 + \varepsilon$.



Case 4

Case 4:
(1) Try $Y = \beta_0 + \beta_1 X + \varepsilon$.
(2) Significant lack of fit.
(3) Try model $Y = \beta_0 + \beta_1 X + \beta_{11} X^2 + \varepsilon$.
(*Note:* β_1 may be significantly different from zero when residual error term is reduced by taking out $\beta_{11} X^2$. See Chapter 6.)

Figure 2.3. Typical straight line regression situations.

- **`3dRegAna`**: Qualitative Variables (ANCOVA)
  - ★ See latest examples here: http://afni.nimh.nih.gov/sscc/gangc/ANCOVA.html
  - ★ Qualitative variables can also be used
    - ➥ *i.e.,* We're modeling the response amplitude to a stimulus of varying contrast when subjects are either young, middle-aged or old.
    - ➥ X_1 represents the stimulus contrast (quantitative): continuous covariate
    - ➥ Create indicator variables X_2 and X_3 to represent age:
      - ➪ X_2 = 1 if subject is middle-aged
        = 0 otherwise
      - ➪ X_3 = 1 if subject is old (*i.e.*, at least 1 year older than Bob Cox)
        = 0 otherwise
    - ➥ Full Model (no interactions between age and contrast)
      - ➪ $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \varepsilon$

        $E(Y) = \beta_0 + \beta_1 X_1$ *for young subjects*

        $E(Y) = (\beta_0 + \beta_2) + \beta_1 X_1$ *for middle-aged subjects*

        $E(Y) = (\beta_0 + \beta_3) + \beta_1 X_1$ *for old subjects*
    - ➥ Full Model (with interactions between age and contrast)
      - ➪ $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_2 X_1 + \beta_5 X_3 X_1 + \varepsilon$

        $E(Y) = \beta_0 + \beta_1 X_1$ *for young subjects*

        $E(Y) = (\beta_0 + \beta_2) + (\beta_1 + \beta_4) X_1$ *for middle-aged subjects*

        $E(Y) = (\beta_0 + \beta_3) + (\beta_1 + \beta_5) X_1$ *for old subjects*

- **`3dRegAna`**: ANOVA with unequal samples
  - ★ **`3dANOVA2`** and **`3dANOVA3`** do not allow for unequal samples in each combination of factor levels
    - ➥ Can use **`3dRegAna`** to look for main effects and interactions
    - ➥ The analysis method involves the use of indicator variables so it is practical for small for small number (~3) of factor levels
  - ★ Details are in the 3dRegAna manual
    - ➥ method is significantly more complicated than running ANOVA; you *must* understand the math
    - ➥ avoid this, if you can, especially if you have more than 4 factor levels or more than 2 factors
    - ➥ Interactions hard to interpret, and contrast tests unavailable

• Cluster Analysis: Multiple testing correction

★ 2 types of errors in statistical tests

➥ What is H_0 in FMRI studies?

➥ Type I = P (reject H_0|when H_0 is true) = false positive = p value

Type II = P (accept H_0|when H_1 is true) = false negative = β

➥ Usual strategy: controlling type I error

(power = 1- β = probability of detecting true activation)

➥ Significance level = α: $p < \alpha$

★ Family-Wise Error (FWE)

➥ Birth rate H_0: sex ratio at birth = 1:1

➢ What is the chance there are 5 boys (or girls) in a family?

➢ Among100 families with 5 kids, expected #families with 5 boys =?

➥ In fMRI H_0: no activation at a voxel

➢ What is the chance a voxel is mistakenly labeled as activated (false +)?

➢ Multiple testing problem: With n voxels, what is the chance to mistakenly label at least one voxel? *Family-Wise Error:* $\alpha_{FW} = 1-(1- p)^n$ -->1 as n increases

➢ Bonferroni correction: $\alpha_{FW} = 1-(1- p)^n \sim np$, if $p << 1/n$

Use $p= \alpha/n$ as individual voxel significance level to achieve $\alpha_{FW} = \alpha$

• Cluster Analysis: Multiple testing correction

- Multiple testing problem in fMRI: voxel-wise statistical analysis
  - ➥ Increase of chance at least one detection is wrong in cluster analysis
- ★ Two approaches
  - ➥ Control FWE: $\alpha_{FW} = P$ (≥ one false positive voxel in the whole brain)
    - ➢ Making α_{FW} small but without losing too much power
    - ➢ Bonferroni correction doesn't work: $p=10^{-8}\sim10^{-6}$
      - *Too stringent and overly conservative: Lose statistical power
    - ➢ Something to rescue? Correlation and structure!
      - *Voxels in the brain are not independent
      - *Structures in the brain
  - ➥ Control false discovery rate (FDR)
    - ➢ FDR = expected proportion of false + voxels among all detected voxels

- **Cluster Analysis: `AlphaSim`**
  - ★ FWE: Monte Carlo simulations
    - ➥ Named for Monte Carlo, Monaco, where the primary attractions are casinos
    - ➥ Program: **`AlphaSim`**
      - ➢ Randomly generate some number (e.g., 1000) of brains with false positive voxels
      - ➢ See what clusters form by chance alone, given spatial smoothness in data
      - ➢ Parameters:
        - * ROI
        - * Spatial correlation
        - * Connectivity
        - * Individual voxel significacet level (uncorrected *p*)
      - ➢ Output
        - * Simulated (estimated) overall significance level (corrected *p*-value)
        - * Corresponding minimum cluster size
      - ➢ Decision: Counterbalance among
        - * Uncorrected *p*
        - * Minimum cluster size
        - * Corrected *p*

- **Cluster Analysis**: **`AlphaSim`**
  - ★ Example

```
AlphaSim \
-mask MyMask+orig \
-fwhmx 4.5 -fwhmy 4.5 -fwhmz 6.5 \
-rmm 6.3 \
-pthr 0.0001 \
-iter 1000
```

Program

Restrict correcting region: ROI

Spatial correlation

Connectivity: how clusters are defined

Uncorrected *p*

Number of simulations

  - ★ FWHM are estimated using 3dFWHM: see http://afni.nimh.nih.gov/sscc/gangc/mcc.html
  - ★ Output: 5 columns
    - ➥ Focus on the 1st and last columns, and ignore others
    - ➥ 1st column: minimum cluster size in voxels
    - ➥ Last column: alpha (α), overall significance level (corrected *p* value)

Cl Size	Frequency	Cum Prop	p/Voxel	Max Freq	Alpha
2	1226	0.999152	0.00509459	831	0.859
3	25	0.998382	0.00015946	25	0.137
4	3	1.0	0.00002432	3	0.03

    - ➥ May have to run several times with different uncorrected *p*:
      - ➪ increase uncorrected p --> increase in minimum cluster size

• Cluster Analysis: 3dFDR

	Declared Inactive	Declared Active	
Truly Inactive	N_{ii}	N_{ia} (I)	T_i
Truly Active	N_{ai} (II)	N_{aa}	T_a
	D_i	D_a	

★ Definition:

FDR = proportion of false + voxels among all detected voxels

$$FDR = \frac{N_{ia}}{D_a} = \frac{N_{ia}}{N_{ia} + N_{aa}}$$

★ Doesn't consider

- ➥ spatial correlation
- ➥ cluster size
- ➥ connectivity

★ Again, only controls the **expected** % false positives among declared active voxels

★ Algorithm: statistic (*t*) ➔ *p* value ➔ FDR (*q* value) ➔ *z* score

★ Example:

```
3dFDR -input 'Group+tlrc[6]'      \
   -mask_file mask+tlrc           \
   -cdep -list                    \
   -output test
```

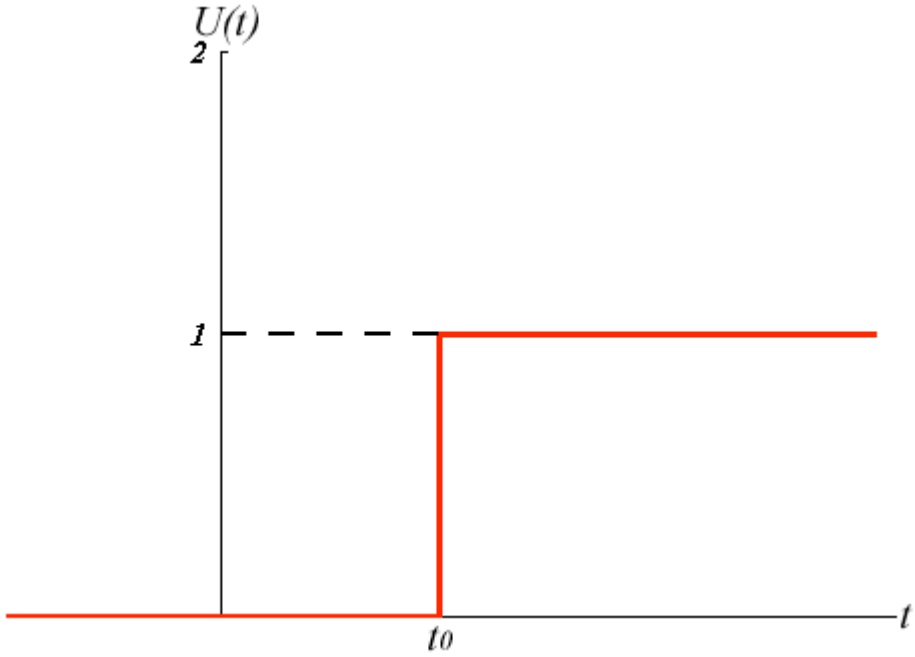
- One statistic
- ROI
- Arbitrary distribution of *p*
- Output

- **Cluster Analysis**: FWE or FDR?
  - ★ Correct type I error in different sense
    - ➥ FWE: $\alpha_{FW} = P$ (≥ one false positive voxel in the whole brain)
      - ➢ Frequentist's perspective: Probability among many hypothetical activation brains
      - ➢ Used usually for parametric testing
    - ➥ FDR = expected % false + voxels among all detected voxels
      - ➢ Focus: controlling false + among detected voxels in one brain
      - ➢ More frequently used in non-parametric testing
  - ★ Fail to survive correction?
    - ➥ At the mercy of reviewers
    - ➥ Analysis on surface
    - ➥ Tricks
      - ➢ One-tail?
      - ➢ ROI – (the partial truth and nothing but the partial truth, so help you God)?
    - ➥ Many factors along the pipeline
      - ➢ Experiment design: power?
      - ➢ Filtering FWHM and minimum cluster size
      - ➢ Poor spatial alignment among subjects

- **Cluster Analysis**: Conjunction analysis
  - ★ Conjunction analysis
    - ➥ Common activation area
    - ➥ Exclusive activations
  - ★ Double/dual thresholding with AFNI GUI
    - ➥ Tricky
    - ➥ Only works for two contrasts
    - ➥ Common but not exclusive areas
  - ★ Conjunction analysis with `3dcalc`
    - ➥ Flexible and versatile
    - ➥ **Heaviside unit (step function)**

      defines a *On/Off* event

$$U(t-t_0)=\begin{cases}1 & t \geq t_0\\ 0 & t < t_0\end{cases}$$



- **Cluster Analysis**: Conjunction analysis
  - ★ Example with 3 contrasts: A vs D, B vs D, and C vs D
    - ➥ Map 3 contrasts to 3 numbers: A > D: 1; B > D: 2; C > D: 4 (why 4?)
    - ➥ Create a mask with 3 subbricks of *t* (all with a threshold of 4.2)

```
3dcalc -a func+tlrc'[5]' -b func+tlrc'[10]' -c func+tlrc'[15]' \
-expr 'step(a-4.2)+2*step(b-4.2)+4*step(c-4.2)' \
-prefix ConjAna
```

    - ➥ 8 (=2^3) scenarios:

      0: none;

      1: A > D but no others;

      2: B > D but no others;

      3: A > D and B > D but not C > D;

      4: C > D but no others;

      5: A > D and C > D but not B > D;

      6: B > D and C > D but not A > D;

      7: A > D, B > D and C > D

• Miscellaneous

- ★ For more information on
  - ➥ Fixed-effects analysis
  - ➥ Sphericity and Heteroscedasticity
  - ➥ Trend analysis
  - ➥ Correlation analysis (aka functional connectivity)

see http://afni.nimh.nih.gov/sscc/gangc

- **Need Help?**
  - _ Command with “-help”
    - **`3dANOVA3 -help`**
  - _ Manuals
    - http://afni.nimh.nih.gov/afni/doc/manual/
  - _ Web
    - http://afni.nimh.nih.gov/sscc/gangc
  - _ Examples: HowTo#5
    - http://afni.nimh.nih.gov/afni/doc/howto/
  - _ Message board
    - http://afni.nimh.nih.gov/afni/community/board/
  - _ Appointment

➢ **Contact us @1-800-NIH-AFNI**

Further Directions for Group Analysis Research and Software

- In a mixed effects model, ANOVA cannot deal with unequal variances in the random factor between different levels of a fixed factor
  - ★ Example: 2-way layout, factor A=stimulus type (fixed effect), factor B=subject (random effect)
    - ➥ As seen earlier, ANOVA can detect differences in means between levels in A (different stimuli)
    - ➥ But if the measurements from different stimuli **also** have significantly different variances (*e.g.*, more attentional wandering in one task vs. another), then the ANOVA model for the signal is wrong
    - ➥ In general, this "heteroscedasticity" problem is a difficult one, even in a 2-sample *t*-test; there is no exact *F*- or *t*-statistic to test when the means and the variances might differ simultaneously
- Although ANOVA does allow somewhat for intra-subject correlations in measurements, it is not fully general
  - ★ Example: 2-way layout as above, 3 stimulus types in factor A; general correlation matrix between the 3 different types of responses is but ANOVA only properly deals with the case $\rho_{12}=\rho_{13}=\rho_{23}$ (recall we are assuming subject effects are random; this is the correlation matrix for the intra-subject random responses).

$$\begin{bmatrix} 1 & \rho_{12} & \rho_{13} \\ \rho_{12} & 1 & \rho_{23} \\ \rho_{13} & \rho_{23} & 1 \end{bmatrix}$$

- Possible solution: general linear-quadratic minimum variance mixed effects modeling
  - ★ A statistical theory not yet much applied to FMRI data (but it will be, someday)
  - ★ Questions of sample size (number of subjects needed) will surely arise